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ORALLY ADMINISTERED ANABU™ (GlcNBu) PROTECTS JOINT SURFACES IN STREPTOCOCCAL CELL WALL ANTIGEN (SCW)-INDUCED ARTHRITIS IN LEWIS RATS

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In previous work we had shown that GlcNBu (N-butyryl glucosamine) decreases the inflammation of SCW arthritis and increases BMD in this model. This investigation explores the effects of GlcNBu on bone loss.
Chronic SCW arthritis was induced in female rats by a single IP injection. The Groups studied were: (a) no arthritis, no treatment; (b) arthritis, no treatment; (c) arthritis, GlcNBu 20mg/kg/day; (d) arthritis, GlcNBu 200mg/kg/day. The removed left tibiae were scanned using micro computed tomography to qualitatively and quantitatively measure the GlcNBu dosage effects on bone architecture.
Isosurfaces generated from the scans show progressively less bone erosion with increasing dosages of GlcNBu.(Figure). Trabecular bone volume, number and thickness, and subchondral plate thickness increased with GlcNBu dosage, so that Group (d) was similar to Group (a). Significant increases occurred predominantly in the lateral epiphysis and the metaphysis of the high dose group. The structure model index values decreased significantly ($p=0.030$) in the metaphysis from the untreated SCW-induced group to the high dose group, indicating a less rod-like trabecular structure. Strut analysis showed a significant increase ($p=0.034$) in the length of node to node struts from the untreated SCW-induced group to the high dose group on the medial side of the proximal epiphysis.

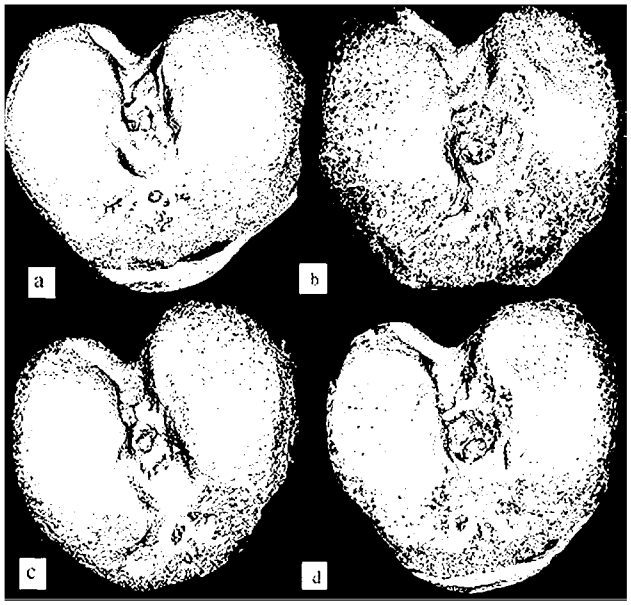

Fig. 1. Isosurfaces of proximal rat tibia (a) No arthritis, no treatment; (b) arthritis, no treatment; (c) arthritis + low dose GlcNBu; (d) arthritis + high dose GlcNBu.

The results indicate a dose-dependent increase in bone connectivity. The metaphyseal bone structure changes suggest that GlcNBu functions systemically. Comparison of the isosurfaces and the architectural parameters studied in this investigation demonstrate that GlcNBu effectively protects bone from further erosion in this model of chronic inflammatory arthritis.

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CHONDROITIN SULFATE EXERTS BENEFICIAL EFFECTS ON THE MECHANISMS LEADING TO OSTEOARTHRITIS SUBCHONDRAL BONE REMODELING

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Aim of study: In osteoarthritis (OA), the increased subchondral bone remodeling is associated with the development of cartilage lesions. These changes are related to an altered metabolism of the osteoblasts. The aim of this study was to determine the effect of chondroitin sulfate on the expression/production of the major osteoblast factors involved in the remodeling of human OA subchondral bone.
Methods: The effect of chondroitin sulfate (200 μg/ml) on human OA subchondral bone osteoblasts, before and after stimulation with vitamin D_3 ($1,25(OH)_2D_3$; 50 nM), was measured on the major phenotypic factors, alkaline phosphatase, and osteocalcin; on the inflammatory mediators, IL-6 and COX-2; and on the bone remodeling factors, RANKL and OPG.
Results: Data showed that the level of alkaline phosphatase activity upon stimulation by vitamin-D_3 challenge, was increased by a 1.6-fold over basal values. Chondroitin sulfate did not affect basal and vitamin D_3-induced alkaline phosphatase or osteocalcin release. On the metabolic factors, chondroitin sulfate had no significant effect on IL-6, but significantly inhibited either basal or vitamin D_3-stimulated COX-2. In the presence of vitamin D_3, chondroitin sulfate upregulated OPG expression and production. On the protein level, chondroitin sulfate significantly increased OPG in both basal conditions and in the presence of vitamin D_3. RANKL expression was almost abrogated with chondroitin sulfate under basal conditions. Interestingly, under basal conditions, chondroitin sulfate significantly upregulated the expression ratio of OPG/RANKL. Vitamin D_3 decreased this ratio, but chondroitin sulfate in the presence of vitamin D_3 reversed this decrease.
Conclusions: Our data indicate that chondroitin sulfate does not appear to overly affect cell integrity or osteoblast phenotypic cell markers. However, chondroitin sulfate by increasing the ratio of OPG/RANKL could exert a positive effect on OA subchondral bone structural changes, indicating a potential direct beneficial effect. Indeed, the expression of RANKL is increased in abnormal osteoblasts, thereby weighting the balance of OPG/RANKL toward bone destruction. Consequently, our data are of major significance as it is the ratio OPG/RANKL that dictates the magnitude of osteoclastogenesis. These findings may help explain the mechanisms by which this drug could exert its effect on the progression of OA structural changes.

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EFFECT OF SOME ANTHRAQUINONES ON TRABECULAR AND SUBCHONDRAL BONE IN AN EXPERIMENTAL ANIMAL MODEL

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Aim of the Study: GR373, GR 375, and GR 377 are patented anthraquinone derivatives which are active on both cartilage and bone. The water soluble GR 373 (anthraquinone 2,6-disulfonic

acid) resulted more active as a chondroprotector. The GR 375 and GR 377, which are respectively the N,N-diethyl and diphenetidin 2,6 disulfonamide derivatives of GR 373, are more active on bone. In this research we investigate the effect of GR 375 and GR 377 on trabecular and subchondral bone in experimental animal.
Methods: 18 female Wistar rats having a body weigth of 180±10 g were subjected to an experimentally-induced osteoporosis by surgically removing the ovaries. The animals were divided into three groups as follows: 1) control, 2) treated with GR 375 25 mg/kg/os and 3) treated with GR 377 at the same dosage. After a three-month treatment period the rats were sacrificed and their posterior legs removed and stored in formalin solution at pH 7.2. They were then dehydrated with warm methyl alcohol under vacuum and methyl methacrylate embedded. Frontal 100 and 20-30 μm thick sections of the specimens were prepared to evaluate the effect of treatment. Microradiographs of the femoral head and neck region were taken. The trabecular bone volume (%TBV) was measured histologically. The results indicate the treated animals (GR 377: 54.53 ± 7.37, GR 375: 49.48 ± 9.97) to have a greater %TBV when compared to the control group (41.32 ± 4.54), nonetheless statistical significance was obtained only for the GR 377 treated group. Similar results were obtained after having assessed the tibial trabecular thickness. The subchondral and cortical components of bone were also markedly increased in the treated animals when compared to their control counterparts.Bone density and mineralization are statistically higher in treated animals.
Conclusion: 2,6 diphenetidine anthraquinone disulphonamide (GR 377) has been shown to be effective in increasing bone mass in the trabecular, subchondral and cortical components of bone in the ovariectomized rat. N,N' diethyl 2,6 anthraquinone disulphonamide (GR 375) was also shown to be effective, although not to the same extent as that of GR 377. Other studies have demonstrated these compounds to significantly decrease IL-6 and iNOS in LPS-challenged rats and to modulate the expression of some proteins in bone metabolism and mineralization. The data suggest that one mechanism of GR 377 and GR 375 action is the modulation of bone gene expression.

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ORAL ADMINISTRATION OF ANABU™ (GlcNBu) TO OVARIECTOMIZED (OVX) RATS INCREASES BONE MINERAL DENSITY (BMD), LONG BONE GROWTH AND MODULATES GENE EXPRESSION IN THE LIVER

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In previous studies, we had shown increased BMD in the long bones of an inlammatory arthritis model in the rat, by feeding GlcNBu (N-butyryl glucosamine). The aim of this study is to determine if oral admininistration of GlcNBu affects BMD, bone growth and gene expression in the non-OVX and the OVX-rat model.
Female Sprague Dawley rats were randomized into four Groups (8 animals each). In addition to their normal diet, the animals were fed on a daily basis (once a day), with either of GlcNBu 200 mg/kg, or an equimolar amount of glucose (Glc) for 6 months. Groups 1, 2, 3 and 4 were, respectively: Glc-fed, non-OVX, (Control); GlcNBu-fed, non-OVX; Glc-fed, OVX; GlcNBu-fed, OVX. The BMD and body composition was measured by DXA (Hologic 4500, small animal software) every 2 months. At sacrifice (6 months), femurs were removed for physical measurements. The total RNA from the liver was extracted (RNeasy Kit,Qiagen) and gene expression was assessed by microarray (rat 8K gene, fluorescent Cy3 and Cy5), with Group 1 serving as the control for each of Groups 2-4.
The results showed that there was increased length of femurs for Group 4 vs 3 and in femoral wet weights (trends), as well as for Group 2 vs 1 (Fig. 1). As expected, OVX led to increased body mass. The BMD of Group 4 femoral heads were higher than Group 3. Total body mineral content + lean body mass was also highest in Group 4.

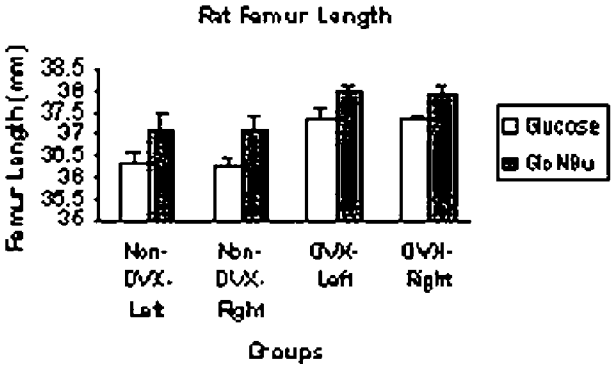

Fig. 1. Effect of GlcNBu on femoral length (OVX and Controls).

By microarray (all compared to Group 1), the number of regulated genes (> 2-fold) in the liver were: Group 2, 13; Group 3, 40; Group 4, 57. A small number of genes were highly regulated only in Group 3.
We conclude that oral administration of GlcNBu results in enhanced growth and increased BMD of femurs in the OVX rat. The microarray analysis (rat 8K gene chip) showed that a number of genes in the liver were remarkably regulated by GlcNBu. Comparisons of the 4 experimental Groups suggest that GlcNBu feeding selectively regulates some genes post-ovariectomy. Since some of the selectively up-regulated genes in Group 4 encode for cirulating protein growth factors, we speculate that this may constitute a mechanism of action for the effects of GlcNBu on bone.

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EXPRESSION OF HIF-1α AND FACILITATIVE GLUCOSE TRANSPORTERS (GLUTs) IN NORMAL AND DEGENERATE HUMAN INTERVERTEBRAL DISCS

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Aim of Study: Tissues such as cartilage and the intervertebral disc (IVD) are hypoxic and avascular and this affects glycolysis and cell metabolism. We have recently shown that human chondrocytes express the hypoxia-inducible transcription factor alpha (HIF-1α), along with a number of facilitative glucose transporters including GLUT1, GLUT3 and GLUT9. Other groups have shown that cells from the rat nucleus pulposus (NP) express both GLUT1 and HIF-1α, yet to date, no studies have identified the expression of HIF-1α or other GLUTs in the human intervertebral disc. In this study we have used immunohistochemistry (IHC) to examine the expression of HIF-1α, GLUT1, GLUT3 and GLUT9 in normal and degenerate IVDs.
Methods: Thirty-one paraffin-embedded human intervertebral disc biopsies were chosen for analysis. These included 10 non-degenerate and 21 degenerate discs. IHC was conducted using antibodies against HIF-1α, GLUT1, GLUT3 and GLUT9 and human tissue microarrays served as positive controls.
Results: IHC confirmed that HIF-1α and GLUTs 1, 3 and 9 proteins were all expressed in the normal human IVD. Expression